between proteins and sugars, peptides or nucleic acids, which may be useful to study catalysis by enzymes such as amylases, cellulases, proteases or nucleases. This new generation of substrates holds the promise of becoming a key tool to study single-bond chemistry under force.

59-Plat
Single-Molecule Analysis of the Recognition Forces Underlying Nucleo-Cytoplasmic Transport
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Macromolecular exchange between the nucleus and cytoplasm of cells is gated at nuclear pores by a family of intrinsically-disordered nucleoporins (nups). These feature phenylalanine-glycine repeats in 'cohesive' domains (FG domains) that interact to form a physical barrier. Through unknown mechanisms, karyopherins (importins, exportins, transportins, NTRs) penetrate this barrier to facilitate the movement of large proteins and RNPs across without paying an external energetic cost, simply by interacting with FG domains. To address the molecular binding and dissociation mechanisms involved in this coupled gating-translocation process, single molecule force spectroscopy was used here to measure the interaction force between nup FG repeats, and between importin beta and nup FG repeats. As predicted, cohesive FG domains bound each other through multiple FG repeat interactions. In contrast importin bound only relaxed coil multiple FG repeats simultaneously, whereas just one FG binding site was assessed in collapsed coil multiple FG domains. Most importantly, the interaction forces and fast dissociation rate constants measured between two FG repeats, and between importin and one FG repeat, were almost identical. This suggests that the force needed to separate interactions between FG repeats of nups at the NPC (*i.e.* for kaps to penetrate the gate and translocate across) could be provided in full by the enthalpy gained through the formation of karyopherin-FG repeat interactions.

60-Plat
Direct Observation of Catch-Bonds in Focal Adhesions of Living Cells
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Single molecule force spectroscopy data have demonstrated that the chemical bonds between extracellular matrix proteins, integrins and several proteins of the focal adhesion complex show catch-bond behavior: the binding strength increases under mechanical load. It remains unknown, however, whether catch-bond mechanisms are of any relevance for stabilizing matrix adhesions in living cells. To measure adhesion strength, we bind RGD-coated magnetic beads to integrin adhesion receptors of living cells and apply forces of up to 80 nN with a magnetic tweezer. Under mechanical load, the beads detach stochastically from the cell surface, and the characteristic force at which 50% of the beads detach is a measure of the adhesion strength. In the case of a pulling force that increases linearly with time, the characteristic bead detachment force is expected to increase logarithmically with the loading rate for thermally activated Bell-type molecular bonds. We find that the detachment force tends to increase faster than logarithmically, demonstrating that the adhesion bonds strengthen under force. This may be indicative of catch bonds, but could also arise from a complex binding energy landscape that, as it is tilted under a mechanical load, presents different energy barriers against detachment. To distinguish between these two possibilities, we applied a staircase-like mechanical load with the same average loading rate but with forces that at all times exceeded those of the linear ramp protocol. We find significantly increased detachment forces under a staircase-like loading protocol compared to a linear force ramp, which rules out other mechanisms except catch-bond behavior.

61-Plat
Single Molecule Measurements of Catch Bond Formation in Cadherin Cell-Adhesion Proteins
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We resolve the molecular interactions by which cadherin cell adhesion proteins tune the mechanical properties of cell-cell junctions. Cells in multicellular organisms undergo constant rearrangements; during tissue formation and wound healing, cells divide, change positions and tug on their neighbors. While the genes that orchestrate these process have been studied extensively, we do not know nearly enough about how cell-cell contacts resist physical force. At the molecular level, cells are held together by adhesion proteins, most commonly by the cadherin family of proteins. Cadherins are essential for tissue formation and for maintaining tissue integrity. Using single molecule force measurements, we demonstrate that cadherin bonds become stronger as cells are pulled apart. This counter-intuitive data is the first demonstration that cadherin's form catch bonds; the proteins grip strongly in the presence of an external force but detach when force is removed. Our results suggest a molecular mechanism for regulating cell-cell adhesion under conditions of variable mechanical stress.

62-Plat
Direct Measurement of the Diffusion Dynamics of an Extended Poly-Ubiquitin Under Constant Force
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Force Spectroscopy is a technique in which single proteins are probed under mechanical perturbation. The force-length course measured in force spectroscopy is commonly described in terms of a diffusive process over a one dimensional potential of mean force, which reflects the end to end motion of the protein. Accordingly, the diffusion coefficient of a protein or polypeptide over its potential of mean force, D, is a basic property that relates the detected kinetics to the applied load. So far, D had been calculated from measurements taken in bulk, where the molecule is free to diffuse without any applied mechanical loads. The reported values indicate rapid dynamics that cannot explain the substantially slower timescales observed by a collapse trajectory of a single molecule under force. To this end we built a fast AFM apparatus with an improved characteristic time response of ~100 µs. Using this novel setup we pulled on poly-ubiquitin, which has a distinct fingerprint, and then applied a force quench protocol between 250 and 100 pN to probe its recoiling dynamics. We fitted this data with a high force approximation analytical expression, which was verified using Brownian Dynamics, to measure the value of D for the recoiling traces. We report here for the first time an averaged value of D = 1374 ± 222 nm^2/s, which is interestingly about five orders of magnitude smaller than the ones measured in bulk. The value of D measured here accounts for the observed slow recoiling timescales (~1 ms) of a single poly-ubiquitin under mechanical load. Moreover, this value is significant when describing elastic systems where proteins are bound on both sides and still go through conformational changes.

63-Plat
Filamin's Force Sensing Mechanism Revealed by Optical Tweezers
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Cell adhesion, spreading and migration, as it occurs during embryogenesis or wound healing and hemostasis, involve a complex interplay between the extracellular matrix, transmembrane adhesion receptors and the cytoskeleton. A prominent role plays human filamin A, since it is a widely expressed actin cross-linker which additionally binds a multitude of transmembrane receptors such as beta integrins or the von Willebrand Factor receptor GPIbα.
Although it has been shown that beta integrins or GPIbα bind to the Ig-like domain 21 of filamin A, the molecular-level mechanism for filamin binding, especially its regulation, is still unclear. Filamin can auto-inhibit the interaction by hiding the binding site through intra-molecular interactions and it has been suggested that the binding site can be exposed by a force-induced conformational change.
To address the question whether filamin's binding site in domain 21 was activated by force, we adapted a highly sensitive optical tweezers setup to pull at single domain pairs of human filamin A containing the receptor binding site and the auto-inhibiting region. We were able to apply and detect low forces in the physiological relevant regime around 3 pN and monitor the conformational change by measuring the length increase of the protein on a sub-millisecond timescale. This allowed us to distinguish in real-time between the inhibited closed conformation and the active open one. Additionally, we were able to detect the binding of different ligands added in solution and how the applied force increased the binding rate. Therefore, filamin A can be regarded as a force sensor.

64-Plat
Studying the Bacterial Flagellar Motor using an Optical Torque Wrench
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At the biomolecular level, the physical quantity *torque* manifests itself in a number of ways e.g. in the conformational changes of biopolymers such as DNA and in actions of both linear and rotary molecular motors. The optical torque wrench, an optical tweezers setup with the capability of applying and